Pine mycorrhizal communities in pure and mixed pine-oak forests: abiotic environment trumps neighboring oak host effects

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Abstract

Scots pine (*Pinus sylvestris*) is frequently planted as a monoculture, but it is also grown in mixed plantations with other native trees such as pedunculate oak (*Quercus robur*). Both pine and oak form ectomycorrhizas that cover their roots and extend into the soil, facilitating tree water and nutrient uptake in exchange for photosynthetic carbon. Forming the interface between the soil and trees, mycorrhizal fungi are key drivers of biogeochemical cycling in terrestrial ecosystems and play an important role in the successful establishment of tree seedlings. They can, however, be susceptible to changes in the soil environment and in their hosts. Both environment and neighboring hosts affect how fungi colonize roots and may affect their host preference. Despite the importance of mycorrhizal fungi in forest ecosystems, little is known about the biodiversity and functional effects of mycorrhizal communities in mixed compared with monoculture plantations. Changes in mycorrhizal richness and composition can result in changes in functional groups with consequences for forest ecosystem stability and functioning. We compared pine mycorrhizas in eight mixed plantations of pine and oak and eight pine monocultures in two forests in England, and we investigated the main factors driving their taxonomic and functional composition. Geographical location and litter pH explained over 50% of the variation in pine mycorrhizal communities. Different environmental factors affected taxonomic and functional composition across stands, indicating functional redundancy. Pine tended to associate with more fungi in the presence of oak, but the abiotic environment exerted a stronger influence than oak presence on pine mycorrhizal diversity.

47 Keywords *Pinus sylvestris*; *Quercus robur*, monocultures; polycultures;
48 ectomycorrhizas; diversification

1. Introduction

A majority of European plantations are monocultures for producing timber, paper and/or firewood (Evans, 2009; Forest Europe, UNECE and FAO, 2011). There is increasing recognition, however, of the value of planting mixtures despite possible reduced production benefits compared with monospecific plantations. Mixed species plantations, or polycultures, can show greater resilience to pests and pathogens, improved resource use efficiency, higher rates of above-ground biomass production and carbon (C) sequestration, and increased habitat provision for wildlife compared with monocultures (Jactel and Brockerhoff, 2007; Piotta, 2008; Pretzsch and Schütze, 2009; Felton et al., 2010; Richards et al., 2010; Cavard et al., 2011; Haas et al., 2011; Castagneyrol et al., 2013; Mason and Connolly, 2013). Mixed species plantations can additionally reduce the risk of plantation failure due to environmental change; i.e. in accordance with the 'insurance hypothesis', when faced with unpredictable environmental change, resilient species in a mixture will replace the more susceptible species, leading to a reduced risk of loss in productivity (Yachi and Loreau, 1999). Species choice is also recognized as being important to improve adaptation to environmental change, and also for maintaining or enhancing local biodiversity; in this respect, native tree species tend to be recommended for planting over exotic species (Lamb, 1998; Hartley, 2002).

Scots pine (*Pinus sylvestris*) and pedunculate oak (*Quercus robur*) are important European timber species. They are native to Britain where they hold significant conservation and heritage value (White, 1995; Harmer and Morgan, 2007; Hanewinkel et al., 2012). Despite being frequently planted as monocultures, these tree species are also grown successfully as oak-pine polycultures and are often

species of choice for reforestation and conversion efforts across Europe (Zerbe and Kreyer, 2007; Morneau et al., 2008, Del Río and Sterba, 2009; Matos et al., 2010; Guest and Huss, 2012; Mason and Connolly, 2013). Recommendations to plant Scots pine-oak mixtures over monocultures are supported by some evidence of specific biodiversity gains, such as observations of increased diversity and abundance of earthworms in pine-oak mixtures compared with pine monocultures; this evidence base is restricted, however, to only a few studies (Kautz and Topp, 1998; Ammer et al., 2006; Barsoum et al., 2014, 2016). More supporting evidence for this as well as other successful polycultures is needed, especially considering mixed compared with monoculture influences on organisms that play major roles in forest ecosystem functioning.

In this study, we examine and compare the diversity and functional structure of pine ectomycorrhizal (ECM) fungal communities in Scots pine plantations grown as monocultures and in mixtures with pedunculate oak. Both Scots pine and oak roots form ectomycorrhizas. These ancient fungal-plant mutualisms, made up of hyphae covering and extending from plant fine roots, drive biogeochemical cycling in terrestrial ecosystems. In exchange for plant photosynthetic carbon, ECM fungi gather soil nutrients and water for their plant hosts, improving the establishment success of seedlings, plant nutrient status and growth and providing protection against pathogens and drought (Smith and Read, 2008). Ectomycorrhizas form the interface between plant and soil, which makes them susceptible to changes in both soil conditions and the plant host. Despite their dominant influence in the functioning of forests (Clemmensen et al., 2013; Averill et al., 2014; Baskaran et al., 2016, Terrer et al., 2016), there is poor understanding of the functional implications of

differences in ECM community composition between monocultures and mixed stands. A better understanding of the ECM communities that associate with pine in both monocultures and mixed plantations and their environmental threats can help guide reforestation efforts, the management of plantations as monocultures and polycultures, and policy decisions on forest diversification (Cavard et al., 2011; Suz et al., 2015).

Ectomycorrhizal fungi have different soil exploration strategies, based on the extent and production of mycelium and the formation of hyphal structures specialized for long-distance transport of nutrients and water (rhizomorphs). Individual ectomycorrhizas can thus be classified into soil exploration types that reflect not only how these fungi explore the environment, but also the carbon and nitrogen fluxes between both symbiotic partners (Agerer, 2001, 2006; Hobbie and Agerer, 2010); therefore, soil exploration types are a proxy for function. Changes in fungal richness affecting these functional groups may have important consequences for forest ecosystem stability and functioning. The taxonomic and functional structure of Scots pine and oak ECM communities are known to vary with different environmental factors, forest management practices and environmental change (Cox et al., 2010; Jumpponen et al., 2010, Jarvis et al., 2013, 2015; Suz et al., 2014; Rincón et al., 2015; Kyaschenko et al., 2017).

Forests dominated by Scots pine are known to host diverse belowground ECM communities (Cox et al., 2010; Pickles et al., 2010; Jarvis et al., 2013, 2015;), although there are typically fewer ECM species associated with Scots pine compared to pedunculate oak (Jumpponen et al., 2010; Suz et al., 2014; Spake et al.,

2016). Mixtures of both host species are expected to harbor higher richness of ECM fungal taxa and more host-specialist fungi because of increased habitat heterogeneity, modified soil physical conditions and resource niche partitioning that allow the co-occurrence of many species (Bruns, 1995; DeBellis et al. 2006; Ishida et al., 2007; Dickie, 2007; Peay, 2016). Moreover, generalist fungi that can associate with many hosts are also expected to be present in greater numbers in mixed forests (Cavard et al., 2011). However, negative effects of competition and allelopathy among tree hosts might have the opposite effect in mixed plantations (Tedersoo et al., 2014). Changes in host preference composition can influence fungal and plant community dynamics, as well as the structure and functioning of common mycorrhizal networks, with consequences for ecosystem condition and resilience (van der Heijden et al., 2015; Molina and Horton, 2015).

Both the environment and neighboring hosts affect how ECM fungi form mycorrhizas (Molina and Horton, 2015). Some studies have shown a strong influence of host identity on ECM communities (Ishida et al., 2007; Morris et al., 2008; Tedersoo et al., 2008; Smith et al., 2009; Aponte et al., 2010) that seems to increase with phylogenetic divergence of the hosts. Other studies have shown little divergence between host species of the same genus (Erlandson et al., 2016) at least on the dominant species (Suz et al., 2014). However, very few studies (see Cavard et al., 2011 for a review) have focused on the effect of a second host species on the mycorrhizal fungi associated with the roots of one tree species growing separately and together in the same field conditions in different geographical areas.

We aimed to: 1) study the effect of oak on ECM communities associated with pine roots in mixed plantations of oak and pine, and compare these with ECM communities in pine monocultures and, 2) identify the main factors affecting pine taxonomic and functional ECM community richness, evenness and composition across the two stand types (pure and mixed stands), and in two forest regions with different environmental conditions.

We hypothesized that 1) the presence of oak in mixed plantations would increase the richness of mycorrhizal fungi associated with pine and would affect the relative abundance of generalists and pine specialists on pine roots (host neighboring effect), 2) differences in environmental conditions, if they exist, between pure and mixed plantations would also affect the mycorrhizal communities associated with pine roots (ecological specificity), and 3) N availability, soil pH and soil moisture would affect pine mycorrhizal communities in both types of stands as has been shown for Scots pine communities in other studies (e.g. Cox et al., 2010; Jarvis et al., 2013, 2015). We conducted this study in two forest regions to test for consistency of any emerging patterns of variation in pine mycorrhizal communities. We also aimed to identify species or species assemblages characteristic of pure pine and mixed pine and oak stands. This knowledge can inform forest management practices and the design of forest diversification policies.

2. Materials and methods

2.1 Study area

The study was undertaken in two large areas of woodland in England, the New Forest, Hampshire, in southern England (50°47'0N, 1°38'0W) and Thetford Forest, East Anglia, in south-east England (52°27'N, 0°51'0E). These two areas were selected for their contrasting levels of mean annual precipitation, mean annual temperature and atmospheric N deposition (New Forest: 425-1,232 mm³, 6.3°C and 8.2 kg ha⁻¹ yr⁻¹ of nitrogen deposition; Thetford Forest: 391-833 mm³, 9.2°C and 18.3 kg ha⁻¹ yr⁻¹) and the availability of Scots pine-oak mixed and pure pine stands. In both forests, standardized sampling was carried out in eight pure Scots pine stands and eight Scots pine-oak mixtures. The mixed stands comprised intimate Scots pine-pedunculate oak mixtures and had no less than 40% of either tree species (40-60% oak). Each stand was at least 3.5 ha in size; they were located at similar elevation ranges (20-90 m.a.s.l. in the New Forest and 20-40 m.a.s.l. in Thetford) and stand ages ranged from 45 to 85 years. Location and main characteristics of each stand are included in Table 1. Environmental variables recorded in each stand are detailed in Table S1 in Appendix A. Further details of stand characteristics and how environmental variables were collected can be found in Barsoum et al. (2016).

2.2 Sampling strategy

In each stand, a central 50 x 50 m plot was used for mycorrhizal sampling. All trees in the plot were numbered. In the pure stands, ten pines were randomly selected, and from each chosen tree, a transect was traced to the nearest pine. In the mixed stands, five pines and five oaks were randomly selected and a transect was traced from each chosen pine to the nearest oak and from each chosen oak to the nearest pine. Along each transect, four soil cores were collected at evenly-spaced distances between trees using a 2 cm diameter x 25 cm depth soil corer. Depth of the soil

cores was recorded for root density calculations. A total of 40 soil cores were collected per plot and stored in sealed plastic bags at 4°C for up to seven days until processing. Soil was rinsed from the samples using a 0.5 mm sieve. Pine roots were then collected from each soil sample by conducting a five-minute search per soil sample under a dissecting microscope. Pine roots were differentiated from oak roots by their characteristic dichotomous and coralloid branching, thicker diameter and usually darker color. From each sample, the four longest roots were selected, and one live mycorrhiza from one end of each root was sampled for analyses as in Cox et al. (2010), giving a total of 160 mycorrhizas per 50 x 50m plot (10 transects x 4 soil cores x 4 mycorrhizas). Brief descriptions of each mycorrhiza sampled were noted, including the presence of rhizomorphs. Sampling periods were June-July 2011 in the New Forest and November-March 2012 in Thetford Forest.

2.3 Mycorrhizal assessment

Genomic DNA from individual mycorrhizas was isolated using Extract-N-Amp (Sigma), and the internal transcribed spacer (ITS) region of the nuclear rDNA was amplified using the primers ITS1F (Gardes and Bruns 1993) and ITS4 (White et al., 1990). Amplicons were purified using ExoSAP-IT (USB, Cleveland, OH, USA) and sequenced bidirectionally using BigDye3.1 with an ABI 3730 (Applied Biosystems, Foster City, CA, USA). Sequences were edited with Sequencher v. 4.2 (Gene Codes Corp., Ann Arbor, MI, USA). A first identification through BLAST searches in GenBank (Altschul et al., 1990) was carried out to facilitate subsequent alignment by family in SeaView v4.6 (Gouy et al., 2010); once trimmed, sequences were further aligned using the algorithm Q-INS-i implemented in MAFFT v7.164b (Kato, 2002). Resulted alignments were used in Mothur v.1.33.0 (Schloss et al., 2009) to generate

OTUs at a 97% similarity threshold. The selected representative sequence of each OTU was then assigned to a unique SH through local BLAST using the UNITE+INSD dataset v7.1 (2016-08-22 release date) and those not belonging to ECM taxa (Rinaldi et al., 2008; Tedersoo et al., 2010) were removed from further analyses. Soil exploration types were recorded for the ECM fungal taxa following Agerer (2001, 2006), DEEMY (Information System for Characterization and Determination of Ectomycorrhizae; www.deemy.de) and our brief descriptions when needed. We also recorded host preference for each taxon. We defined host specialists as those fungi that associate with a particular host genus or family (*Pinus*, *Quercus*, conifer (Pinaceae) or angiosperm (e.g. Fagaceae) and as host generalists those fungi that do not show preference at any host taxonomic level. We based this classification in previous mycorrhizal studies of *Pinus sylvestris*, *Picea abies*, *Fagus sylvatica*, *Quercus robur* and *Q. petraea* in Europe (Cox et al., 2010; Suz et al., 2014; van der Linde, unpublished), the UNITE database and the checklist of the British and Irish Basidiomycota (<http://www.basidiochecklist.info/>). The type of fructification (epigeous versus hypogeous) was also assigned. A representative sequence of each OTU was submitted to GenBank (accession numbers MF352673 to MF352812). Pine roots collected in each soil core were freeze-dried, and their dry weight recorded for root density calculations.

2.4 Statistical analyses

Differences in the environmental variables between pure and mixed stands and between New Forest and Thetford Forest were assessed using two-way ANOVA. Relationships between environmental variables and geography across the 16 stands were assessed by regression of variables against latitude (Jarvis et al., 2013).

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248 Estimated pine ECM richness (Abundance-based Coverage Estimator, ACE; Chao
249 and Lee, 1992) and shared taxa between pure and mixed stand types and between
250 the two forests were calculated using EstimateS (v 9.1.0; Colwell 2013). Species
251 accumulation curves were constructed in R v3.0.2 (R Core Team, 2013) by sampling
252 soil cores randomly without replacement using 1,000 permutations of the function
253 *specaccum* in the vegan package. Evenness was calculated using Pielou's (1966).
254 A Venn diagram was used to visualize the shared number of ECM species between
255 stand types and between forests using the R package *VennDiagram*. Relative
256 abundance was calculated by dividing the number of mycorrhizas of each taxon by
257 the total number of mycorrhizas in each plot. Relative abundance was also
258 calculated for each genus, lineage and exploration type, and for dominant taxa
259 present in at least four sites.

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261 The normality of environmental variables was tested using Shapiro and Wilk (1965).
262 All variables, except litter pH, were Box-Cox transformed. Square root transformation
263 was applied for humus depth and soil K content, and the inverse transformation was
264 used for soil Ca content. Diversity measures were Box-Cox transformed when
265 needed and proportions of the soil exploration types were log transformed.

266

267 Two-way ANOVAs were also used to test for differences in estimated ECM richness
268 and evenness between forests, between stand types and their interactions, and for
269 differences in relative abundance of ECM, grouped by host preference. The
270 simultaneous effects of environmental variables on ECM estimated richness and
271 evenness were assessed using generalized linear regression models with Poisson

error distribution (logarithmic link function) and Gaussian error distribution, respectively. Selection of the explanatory variables was performed using the function *regsubsets* in the *leaps* R package based on the models with the highest statistic R^2 and the function *bestglm* in the *bestglm* R package. Continuous variables with observations from all sites (n=16) were included in the analyses, except geographical coordinates.

To explore the main factors involved in community dissimilarities among sites, NMDS was run fitting 14 environmental variables available for the 16 plots (elevation, latitude, longitude, soil moisture, litter and humus depth, litter pH, pH of the mineral layer, soil potassium (K) content, total N, soil Ca, C/N ratio and soil and stand type) using the *envfit* function in the *vegan* package. Bray–Curtis and Euclidean dissimilarity matrices were generated for community and environmental data, respectively. Square root transformation was used for community data. Singletons were removed from the dataset to reduce the influence of rare species, and the ordination was repeated for dominant taxa and for genus, lineage and exploration type composition. Alternatively and based on its previously reported influence in ECM communities (Peay et al., 2011; Suz et al., 2014) we run an additional NMDS ordination including root density (n=15) and explored its importance as explanatory variable of richness and evenness.

To further assess the variables explaining variation in community composition, automatic selection of variables by permutation tests was carried out using the function *ordistep* in the *vegan* R package. The selected significant variables were used in permutational ANOVA using the R function *adonis*. The subset of

environmental variables used in these analyses was selected based on the significance obtained in the NMDS ordination.

Species indicator analysis (Dufrene and Legndre 1997; De Cceres et al., 2012) was run for the two stand types and the two forests using the functions *multipatt* and *combinespecies* for individual and two-species combinations in the package *indicspecies*. Only dominant ECM taxa were tested.

3. Results

3.1 Environmental variables

Field layer cover percentage was the only variable tending to differ ($p < 0.1$, not shown) between pure and mixed stands ($81 \pm 20\%$ in pure versus $58 \pm 31\%$ in mixed stands). However, New Forest stands showed significantly higher soil C and K contents, C/N ratio, humus depth, soil moisture and root density ($p < 0.05$) than stands in Thetford Forest. Soil P content ($p < 0.05$) and pH of the mineral horizon ($p < 0.1$, not shown) were higher in Thetford stands (Fig. 1).

3.2 Taxonomic composition of pine ECM communities

We collected 2,560 mycorrhizae and we obtained DNA sequences from 1,787 (ca. 70%), of which 1,667 (ca. 93%) corresponded to known ECM fungi. A total of 140 OTUs were obtained at a 97% similarity threshold and assigned to a unique species hypothesis (SH) in the UNITE+INSD database (Kljalg et al., 2013). Seventy-two OTUs (51%) were identified to species, 48 only to genus, 18 to family and two to order. Forty-one OTUs were singletons (present as a single root). Relative

abundance of each OTU per stand in each forest is included in Table S2. Overall, 78% of the pine mycorrhizas collected in both forests were colonized by fungi belonging to Basidiomycota and 22% to Ascomycota. The most abundant family was Russulaceae (47% of all mycorrhizas), followed by Elaphomycetaceae (16%), Atheliaceae (6%) and Thelephoraceae (5%). The Elaphomycetaceae was only found in the New Forest, where it was also the most abundant family (36%), followed by Russulaceae (32%), Cantharellaceae (5%) and Clavulinaceae (4%). In Thetford Forest, the dominant family was Russulaceae (59%) followed by Atheliaceae (8%), Thelephoraceae (7%) and Cortinariaceae (4%). Percentage of colonization by the main ECM families found in pure and mixed stands in each forest is shown in Fig. S1 in Appendix A. Overall, the number of different ECM associated with pine roots per stand ranged from 8 to 31. There were a total of 70 ECM fungi in the New Forest and 88 in Thetford; from these, only 18 were found in both forests.

3.3 Influence of oak on pine ECM communities

Across the two forests, the average estimated ECM richness in pure stands (26 average, 74 total) tended to be lower ($p=0.06$, Fig. 2A) than in mixed stands (38 average, 115 total). We recovered 88% and 73% of the estimated ECM fungi, respectively (Fig. 2B). Mixed stands in Thetford Forest tended to be more even than pure stands ($p=0.09$), while in the New Forest, pure and mixed stands were similarly even (data not shown). Forty-nine ECM were found in both types of stands, 25 were unique to pure pine stands and 66 were unique to mixed stands (Fig. 3A). No ECM fungus occurred in every stand. Six ECM fungi were found in pure and mixed stands in both forests (Fig. 3B) and included the generalists *Russula ochroleuca* (colonizing 15% of the total roots), *Amanita rubescens* (1.5%) and *Thelephoraceae* sp.1

(*Tomentella albomarginata*-like, 1%), and the pine specialists *Lactarius hepaticus* (7%), and *Tylospora* sp. 2 (2%).

There were no differences between stand types in the average proportions of generalists and of conifer tree specialists associated with pine roots; however, pure pine stands tended to show a higher proportion of pine specialists (e.g. *Lactarius hepaticus*, *Russula caerulea* and *Russula recondita*) ($p=0.09$) and mixed stands a higher proportion of angiosperm specialists ($p=0.06$). Nine oak specialists (e.g. *Lactarius quietus*, *Boletus subtomentosus* and *Cortinarius decipiens*) were rarely detected (one to four roots per species across stands) in mixed stands, and two oak specialists were detected in five roots in two pure stands (Fig. S2A&B). The classification of the ECM taxa by host preference is shown in Table S3.

Short-distance mycorrhizas were more abundant in mixed than in pure stands ($p=0.017$, Fig. S3), but there were no differences in the relative abundances of other ECM exploration types between pure and mixed stands. There were no differences between pure and mixed stands in the proportion of fungi producing epigeous or hypogeous fruitbodies.

3.4 Influence of environmental variables on taxonomic and functional composition of pine ECM communities

Estimated richness across plots was best explained by eight variables commonly identified by both variable selection methods used: elevation, soil moisture, litter depth and pH, pH of the mineral layer, and C ($p<0.05$), K and C/N content ($p<0.1$). Regarding evenness, eleven variables were selected in the model as best predictors

using *regsubsets*, but only Ca, total N, C, and C/N ratio were significant ($p < 0.05$) (Table S4). Root density ($n=15$) was selected as predictor of evenness but was not significant once included in the generalized linear regression model (data not shown). Latitude and litter depth explained 56% of the variation in the community data according to *Adonis*; total N was chosen in the automatic selection of variables in the reduced model to explain variability in community composition among stands ($p < 0.1$) but was not significant ($p=0.2$) in the PERMANOVA analysis (Table S5).

The NMDS ordination showed that geographical distance (longitude and latitude), litter and humus depth, litter pH, pH of the mineral layer, K and Ca, C/N ratio and soil type had a significant influence on dissimilarities among ECM communities (Fig. 4A&B). The pine communities in both pure and mixed stands were more similar within forests than between forests, but the type of stand was not significant. The same environmental factors were significant for the whole communities, excluding singletons, as for the dominant fungi (data not shown). Similar results were obtained for the NMDS with genus and lineage matrices. In the latter, C/N ratio was also a significant driver (data not shown). In the NMDS analysis with soil exploration type composition, soil N was significant while soil type, litter depth, soil Ca and C/N ratio were not (Fig. 4C&D). When including root density in alternative NMDS ordination analyses ($n=15$), it was a significant driver of ECM composition at all taxonomic levels tested but it was not a significant for soil exploration type composition (data not shown).

The average proportion of conifer specialists was higher in the New Forest ($p=0.001$, data not shown). In Thetford, the relative abundance of contact types was higher

($p < 0.001$) while medium-distance smooth types ($p = 0.016$) were more abundant in the New Forest (data not shown). We found more fungi producing epigeous fruitbodies in Thetford and more forming hypogeous fruitbodies in the New Forest.

Results of the single indicator and up to two-species combination indicator analyses are detailed in Table 2 and in Table S6, respectively. Across forests, no ECM fungus emerged as an indicator of stand type. However, the combination of the pine specialist *Tylospora* sp. 2 and the generalist *Thelephoraceae* sp. 1 emerged as an indicator of mixed forests (Table S6). No combination indicator emerged for pure stands. In the New Forest, *Russula caerulea* and six combinations of two ECM fungi were indicators of pure stands, while two single species and six combinations of species were indicators of mixed stands. In Thetford Forest, two single species and five combinations of species emerged as indicators of pure stands, while two single species were indicators of mixed stands, i.e. *Thelephoraceae* sp. 1 (*Tomentella albamarginata*-like) and *Russula* sp. 1 (*Russula amoenolens*-like). Lastly, across all pure and mixed stands, six single species (e.g. *Elaphomyces granulatus*, *Russula caerulea* and *Craterellus tubaeformis*) and 22 species combinations were indicators of the New Forest, and three single species (e.g. *Amphinema* sp. 1) and six combinations of two species were indicators of Thetford Forest.

4. Discussion

4.1 Host neighboring effects: influence of oak on pine ECM

We recovered a significant proportion of the estimated total ECM taxa per type of stand (88% and 73% in pure and mixed stands, respectively) across forests (Fig. 2B). In pure stands, we found a total of 74 ECM taxa with an average of 26 taxa associated with pine roots per stand (Fig. 2). This agrees closely with other studies of ECM communities in Scots pine dominated forests; for instance, Cox et al. (2010) found an average of 24 ECM in twelve 0.25 ha stands across Britain and Germany, while in Scotland, Pickles et al. (2010) found 24 ECM in a 120 yr old plantation of Scots pine, and Jarvis et al. (2013) detected up to 20 ECM per stand across 15 pine stands of 0.75 ha and over 100 years old. In our study, the number of ECM associated with pine roots tended to be considerably higher when pine was mixed with oak in plantations (Fig. 2); we detected 115 ECM fungi associated with pine roots with an average of 38 fungi per stand when oak was present. Temperate oaks are known to harbour much greater ECM diversity than Scots pine (Cox et al., 2010; Suz et al., 2014); therefore, the total ECM richness in mixed stands is expected to be even higher if ECM colonising oak roots were also considered.

A complex of mechanisms spanning from competitive and synergistic interactions among fungi, phylogenetic and physiological differences among hosts, and preferential nutrient and water allocation between symbiotic partners might trigger fungal-plant preference and specificity (Dickie, 2007; Tedersoo et al., 2010; Kiers et al., 2011). In the presence of oak, there were fewer pine specialist species (20 ECM fungi in pure vs. 18 in mixed plantations), and at lower abundances, colonizing pine roots (Fig. S2). We did not find significant differences in root colonization by generalist fungi between pure and mixed stands, but on average there were more generalist ECM taxa in mixed stands compared to monocultures (37 and 28 species,

respectively). Multihost fungi are common in mixed stands (Horton and Bruns, 1998; Kranabetter et al., 1999; Kennedy et al., 2003; Rumberger et al., 2004; Roy et al., 2008). These generalist fungi can connect pines and oaks through common mycorrhizal networks, allowing for ECM colonization subsidized by established neighbors, and possibly some translocation of C and/or nutrients between hosts. Compatibility between common mycorrhizal networks between hosts can play a role in restructuring communities when multiple hosts are present (Molina and Horton, 2015). In fact, a nurse effect of pine on oak saplings in mixed plantations has been reported in Britain and Ireland (Kerr et al., 1992; Guest and Huss, 2012). These changes in the ratios of generalist and specialists between stand types might also be driven by their different efficiencies in resource allocation to the host (e.g. Gorissen and Kuyper, 2000).

We also found that in Thetford forest, pine ECM communities tended to be more even in the presence of oak. This, although not consistent across forests, matches previous studies. For instance, Jones et al. (1997) found that the evenness of ECM on Douglas fir was higher when planted with paper birch due to the increased abundance of fungi able to colonize both hosts. The higher number of generalist fungi in mixed stands despite the same proportion of roots colonized by them in mixed and pure stands, can explain the more even ECM communities in mixed stands in Thetford Forest. We only measured pine root density, we did not measure oak root density; lower total root densities could be expected in pure stands, leading to less even communities compared to mixed stands due to competition for roots (e.g. Suz et al. 2014) where priority effects (Kennedy et al., 2009) might also play a role. In fact, pine root density was selected as an explanatory variable of community

evenness but it was not significant once included in the generalized linear regression model (data not shown). In the New Forest, we did not find differences in evenness between the two types of stands, probably due to a higher abundance in mixed stands of *Elaphomyces granulatus* (28% of the mycorrhizas) with respect to other ECM fungi, thus decreasing average evenness.

4.2 Ecological specificity: Environmental conditions and pine ECM communities

4.2.1. Taxonomic composition

Both biotic and abiotic factors can affect the ability of fungi and plants to form mycorrhizas, and this is defined as ecological specificity (Harley and Smith, 1983; Molina et al., 1992; Molina and Horton, 2015). We did not find significant differences in the environmental variables measured in pure versus mixed stands within forests, only between the two forests (Fig. 1). Despite comparatively greater between-stand dissimilarities in ECM in Thetford Forest compared with the New Forest, ECM communities were also more dissimilar between forests than between pure and mixed stands within forests (Fig. 4A; Fig. S1). With the exception of Russulaceae, different families were dominant in each forest. In the New Forest, the false truffle *Elaphomyces granulatus* was dominant in all stands. In Thetford, different mushroom-forming ECM were dominant in different stands; for instance, *Russula ochroleuca* and *Lactarius hepaticus* were dominant in three stands, *R. recondita* was dominant in three others and *R. xerampelina* and *Thelephoraceae* sp. 1 dominated one stand each (Table S2).

Environmental variables have been shown to strongly influence pine ECM communities. Cox et al. (2010) found that N availability was the main driver of pine

ECM communities across Britain and Germany, affecting their richness and evenness and leading to shifts in community composition. At a smaller geographical scale, in Scotland, Jarvis et al. (2013) found soil moisture and rainfall as the main drivers of pine community composition while temperature had an influence on exploration type composition. In our study, ordination analysis showed that geographical location together with environmental variables differing between forests like soil type, C/N ratio, humus depth and soil K, all contributed to shape ECM communities. In Thetford, additional variables such as litter, Ca and pH seemed to also influence ECM community composition. These same factors affecting all ECM at the species level (excluding singletons) also seemed to affect the dominant fungi in these communities and the community composition at higher taxonomic levels (genus and lineage). However, when looking at soil exploration types (as a proxy for functional diversity), differences between pine ECM communities between forests were less evident, and the significance of the environmental variables affecting functional composition of the communities changed; for instance, soil type was not significant and soil N became significant (Fig 4D). These results indicate functional redundancy among different fungi at different taxonomic levels in pine ECM communities and the different influence of environmental factors on taxonomic and functional diversity.

Although both forests showed similar taxonomic richness, their communities were remarkably different, i.e. they only shared 18 ECM and only six were found in both pure and mixed stands in both forests (Fig 3). Among all the variables we tested, geographical location (i.e. latitude) and litter pH explain the highest proportion of the variation in the communities associated with pine roots across stands. Latitude

reflects the compositional differences between the two forests in terms of differences in moisture, humus depth, K, C, P and C/N ratios. Dispersal limitations of some of the fungi might also play a role in community dissimilarities (e.g. Peay et al., 2007). This could be the case for the generalist *Elaphomyces* that produces belowground fruiting bodies, and is thus mainly dependent on mammals for excavation and dispersal. *Elaphomyces*' belowground distribution agrees with the distribution of its fruitbodies (nbnatlas.org). The dominance of *Elaphomyces* in the New Forest - a renowned area of ancient woodland pasture that is still actively grazed by ponies, horses and cattle - and its absence in Thetford Forest might be partially explained, together with specific environmental niche requirements, by major differences in grazing pressure. Long-distance dispersal of belowground (e.g. *Rhizopogon* spp.) and some aboveground (e.g. *Suillus* spp.) fungi has been shown to be primarily dispersed through small mammals (Luoma et al., 2003) or deer (Ashkannejhad and Horton, 2006). Bioassays of mammal dung would help to test this hypothesis. In fact, the overall proportion of hypogeous fruitbodies was significantly higher in the New Forest than in Thetford, where ECM fungi forming epigeous fruitbodies dominated. Selected as single species or in two-species combination indicators, fungi belonging to *Amphinema* and Thelephoraceae form resupinate (crust-like) fruiting bodies, while *Elaphomyces* species produce truffles. These types of fruiting bodies are typically overlooked in aboveground inventories. These findings highlight the importance of collecting mycorrhizas to assess ECM diversity.

Litter pH did not differ between stands types and forests but emerged as one of the variables explaining species richness across plots and seemed to drive compositional differences within Thetford. Stands with higher litter pH showed a

higher number of ECM (data not shown). Pine and oak litter and their decomposition products might affect ECM communities, for instance through the retention of scarce nutrients such as N and phosphorus (Conn and Dighton, 2000). The indirect effect of the host through litter quality has been also studied in communities associated with two oak species in the South of Spain (Aponte et al., 2010); in their study, the Ca content of the litter was the most important variable explaining ECM species distribution. Across our stands, soil Ca content and litter pH were positively correlated and influenced community composition in Thetford. Both pH and Ca have a strong influence in fungal richness globally (Tedersoo et al., 2014). In our study, litter pH was also negatively correlated to litter depth, and also influenced community composition in Thetford.

Different environmental factors affected the richness and evenness of pine ECM communities across sites. Elevation, soil moisture, litter depth and pH, soil mineral pH and C content explained the variation in richness among communities, and total N, Ca, C and C/N ratios explained their evenness. Soil nutrients seemed to have a stronger effect on evenness. These factors have been found to affect ECM communities in other studies (e.g. Jarvis et al., 2003, 2005; Morris et al., 2008; Talbot et al., 2014); interestingly, the strong effect of pH on taxonomic richness and the stronger influence of N on evenness and functional composition of ECM communities have been previously observed (Hobbie and Agerer, 2010; Suz et al., 2014).

4.2.1. Functional composition

Except for short-distance exploration types that were more abundant in the presence of oak, the relative proportions of soil exploration types were similar between pure and mixed stands showing the functional redundancy of pine ECM communities regardless of the presence of oak. Differences in the relative abundance of short distance mycorrhizas were driven by *Elaphomyces* in the New Forest, and *Russula* sp. 1 (*R. amoenolens*-like) and *Tylospora* sp. 2 in Thetford. Differences were also found between forests; in Thetford, contact mycorrhizas were more numerous because of the high abundance in most of the stands of the contact exploration types *R. ochroleuca* and *R. xerampelina*. Contact mycorrhizas have been linked to high N deposition due to their ability to uptake N in inorganic forms (Kjller et al., 2012; Hobbie and Agerer, 2010; Suz et al., 2014). While in the New Forest N deposition is 8.2 kg ha⁻¹ yr⁻¹, at Thetford it is 18.3 kg ha⁻¹ yr⁻¹, well above the critical load for N deposition for ECM communities (Pardo et al., 2010; Jarvis et al., 2013; Suz et al., 2014); however, this is not reflected in the N content of its sandy soil, or in a decrease in the abundance of medium fringe types, known to be sensitive to N (Hobbie and Agerer, 2010; Lilleskov et al., 2011).

Due to the large overlap in pine ECM composition in pure and mixed stands within forests, no single species emerged as an indicator of either stand type and only one species combination was found to be a significant indicator for mixed forests (Table 2 and Table S6). The two species that emerged as combined indicators of mixed stands in Thetford were generalists, while the two species that emerged as indicators of the whole forest were pine specialists. In the New Forest, a generalist, one conifer-preferring and one pine specialist were identified as indicators.

5. Conclusions

For taxonomic and functional community composition, local environmental conditions have a stronger influence than the presence of a second host. The presence of oak exerts only a weak influence on the number of ECM fungi and the host specificity composition of the communities in pine roots; in mixed plantations pines harbor more ECM taxa in their roots, including more generalist and angiosperm specialist taxa, to the detriment of pine specialists. Species richness, evenness, taxonomic and functional composition are affected differently and/or by different factors. Geographical location and litter pH explain most of the variability in ECM pine communities across forests.

The minor influence of oak on ECM functional community structure in pine-oak mixtures compared with pine monocultures suggests that, while there are limited gains to be had in this respect by planting pine in mixtures with oak compared with pure pine, there are no negative consequences of adopting either planting strategy on ECM communities and associated functional benefits. Further research is needed to investigate other species mixtures that are able to form stable and productive stands with no negative (and potentially positive) consequences for associated biodiversity and the delivery of functional benefits. Evidence of this kind could contribute to the wider adoption of successful polycultures, which currently lags behind despite the wide advocacy of mixtures over monocultures by government agencies (Nichols et al., 2006).

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Figure legends

Fig. 1 Environmental variables showing significant differences between New Forest (NF) and Thetford forest (TH). Significant codes: $^{\circ}0.1 > P > 0.05$; $*0.05 > P > 0.01$; $**0.01 > P > 0.001$; $***0.001 > P$.

Fig. 2. A) Boxplot showing the differences in the averaged estimated richness (ACE) in pure stands of Scots pine and in mixed stands with pedunculate oak ($F=4.2$, $p=0.06$). B) Species accumulation curves for pure and mixed stands.

Fig. 3. A) Venn diagram showing the number of ectomycorrhizal taxa observed across type of stands and forests. B) Percentage of total colonization by the six shared ectomycorrhizal fungi in pure stands of Scots pine and in mixed stands with pedunculate oak in the New Forest and Thetford forest.

Fig. 4. A&B) Species and C&D) soil exploration type community composition of the 16 plots displayed using NMDS. Stress of the ordination is 0.068 and 0.064, respectively. Significant variables ($p < 0.05$) are shown by arrows whose length is proportional to the strength of the correlation. Significant codes: $^{\circ}0.1 > P > 0.05$; $*0.05 > P > 0.01$; $**0.01 > P > 0.001$; $***0.001 > P$.

Appendix A. Supplementary material.

Supplementary data associated with this article can be found, in the online version,
at XXXX

Table S1. Environmental variables recorded in the forest stands and used in this study. Values are stand averages.

Table S2. Relative abundance of pine ectomycorrhizal fungi in each stand and forest.

Table S3. Host specificity and/or preference assigned to the ectomycorrhizal taxa detected in pine roots in this study based on different sources of information. G: generalist, A: angiosperm (i.e. Fagaceae) specialist, C: conifer (Pinaceae) specialist, P: pine specialist, O: oak specialist. When there is evidence for preference (rather than strict specificity) this is shown between brackets. The van der Linde et al. assignment is based on oak, beech, spruce and pine mycorrhizal surveys across Europe (unpublished data).

Table S4. A) Parameters of the best general linear model with Poisson error distribution explaining estimated species richness across plots. B) Parameters showing the highest R^2 in the model for predicting evenness across plots.

Table S5. A) Results from the automatic selection of variables in the reduced model to explain variability in pine ectomycorrhizal communities across the 16 stands in this

study; B) Relative importance of latitude, litter pH and total soil nitrogen on the pine communities as revealed from the *Adonis* function (PERMANOVA).

Table S6. Single or two species combination selected as indicators of stand type and forest.

Figure S1. Pine root colonization (%) by the main ectomycorrhizal families (>1% total number root tips) in pure and mixed stands in New Forest and Thetford Forest.

Figure S2. A) Relative proportion and, B) Averaged proportion of Scots pine root tips colonized by ectomycorrhizal fungi grouped by host preference in pure and mixed stands across forests.

Figure S3. Mean percentage (%) or root tip colonization by ectomycorrhizal soil exploration types in pure Scots pine and mixed Scots pine and pedunculate oak stands. $.0.1 > P > 0.05$; $*0.05 > P > 0.01$; $**0.01 > P > 0.001$; $***0.001 > P$.

Forest

New Forest

Thetford Forest

Estimated species richness (ACE)

60
50
40
30
20
10

Pure

Type of stand

Mixed

A

B

A

B

A

B

Variable	r2	Pr(>r)	
Elevation	0.1744	0.285	
Latitude	0.9414	0.001	***
Longitude	0.9423	0.001	***
Soil moisture	0.3558	0.061	.
Litter depth	0.587	0.002	**
Humus depth	0.6763	0.001	***
Litter pH	0.768	0.001	***
Mineral pH	0.7083	0.001	***
Soil K	0.7613	0.002	**
Soil total N	0.1756	0.282	
Soil Ca	0.5859	0.004	**
C/N ratio	0.3627	0.047	*
Soil type	0.6877	0.001	***
Stand type	0.0381	0.583	

C

D

Variable	r ²	Pr(>r)
Elevation	0.0585	0.707
Latitude	0.7048	0.003 **
Longitude	0.7173	0.003 **
Soil moisture	0.2143	0.237
Litter depth	0.3779	0.053 .
Humus depth	0.4878	0.017 *
Litter pH	0.5984	0.002 **
Mineral layer pH	0.5246	0.018 *
Soil K	0.5303	0.01 **
Soil total N	0.4941	0.011 *
Soil Ca	0.3245	0.094 .
C/N ratio	0.0629	0.659
Soil type	0.2922	0.149
Stand type	0.1716	0.078 .

Table 1. Main characteristics of the forest stands surveyed in this study

Stand code	Forest	Location	Tree species	Plantation year	Elevation (m)	Soil type
P01_NF2520	New Forests	1°39'0.0" W; 50°50'22.7" N	<i>Pinus sylvestris</i>	1948	35	Surface Water Gley
P02_NF250	New Forests	1°39'42.9" W; 50°50'27.2" N	<i>Pinus sylvestris</i> + <i>Quercus robur</i>	1929	35	Surface Water Gley
P03_NF2512	New Forests	1°39'52.3" W; 50°50'32.4" N	<i>Pinus sylvestris</i>	1927	45	Surface Water Gley
P04_NF2136	New Forests	1°41'45.7" W; 50°53'18.6" N	<i>Pinus sylvestris</i>	1953	90	Surface Water Gley
P05_NF4311	New Forests	1°32'52.6" W; 50°50'53.1" N	<i>Pinus sylvestris</i> + <i>Quercus robur</i>	1950	40	Surface Water Gley
P06_NF4424	New Forests	1°30'57.6" W; 50°49'54.2" N	<i>Pinus sylvestris</i>	1930	20	Surface Water Gley
P07_NF3426	New Forests	1°38'52.1" W; 50°47'33.9" N	<i>Pinus sylvestris</i> + <i>Quercus robur</i>	1930	35	Surface Water Gley
P08_NF1614	New Forests	1°38'21.3" W; 50°55'42.1" N	<i>Pinus sylvestris</i> + <i>Quercus robur</i>	1936	85	Surface Water Gley
P09_TH3522	Thetford	0°51'6.2" E; 52°27'14.9" N	<i>Pinus sylvestris</i>	1937	35	Calcareous Brown Earth

P10_TH3037	Thetford	0°40'15.4" E; 52029'45.2" N	<i>Pinus sylvestris</i>	1930	30	Brown Earth
P11_TH4751 _4738	Thetford	0°52'35.5" E; 52024'50.8" N	<i>Pinus sylvestris</i>	1967	30	Brown Earth
P12_TH3345	Thetford	0°51'15.0" E; 52029'29.0" N	<i>Pinus sylvestris</i>	1932	40	Brown Earth
P13_TH3548	Thetford	0°51'26.2" E; 52026'4.8" N	<i>Pinus sylvestris</i> + <i>Quercus robur</i>	1934	30	Brown Earth
P14_TH3021	Thetford	0°40'2.3" E; 52030'23.3" N	<i>Pinus sylvestris</i> + <i>Quercus robur</i>	1941	25	Brown Earth
P15_TH3324	Thetford	0°51'32.8" E; 52030'4.4" N	<i>Pinus sylvestris</i> + <i>Quercus robur</i>	1935	40	Brown Earth
P16_TH4716	Thetford	0°53'54.8" E; 52025'35.5" N	<i>Pinus sylvestris</i> + <i>Quercus robur</i>	1934	20	Ground Water Gley

Table 2. Species found in Scots pine roots, selected as indicators of forest and stand type (Dufrêne and Legendre, 1997; De Cáceres et al., 2012). Between brackets, host preference of the ECM taxa: G: generalist, P: pine specialist.

	A	B	stat	p-value*
Thetford: pine + oak				
<i>Thelephoraceae</i> sp. 1 (G)	0.7185	1.0	0.848	0.028*
<i>Russula</i> sp. 1 (G)	0.9278	0.75	0.834	0.034*
New Forest				
<i>Elaphomyces granulatus</i> (G)	1.00	1.00	1.000	0.001 ***
<i>Russula caerulea</i> (P)	1.00	1.00	1.000	0.001 ***
<i>Craterellus tubaeformis</i> (G)	1.00	0.75	0.866	0.035 *
Thetford				
<i>Amphinema</i> sp. 1 (P)	1.00	0.75	0.866	0.030 *
<i>Russula</i> sp. 2 (P)	1.00	0.75	0.866	0.031 *

*Significant codes: $0.1 > P > 0.05$; $0.05 > P > 0.01$; $0.01 > P > 0.001$; $0.001 > P$.

A

B

Table S4. A) Parameters of the best general linear model with Poisson error distribution explaining estimated species richness across plots. B) Parameters showing the highest R^2 in the model for predicting evenness across plots.

A)

	Estimate	Std. Error	z value	Pr(> z)
(Intercept)	5.32237375	1.041816636	5.108743	3.243086e-07
Elevation	-0.01616966	0.004180277	-3.868082	1.096947e-04
Soil_moisture_June_Sept	-4.74168449	1.659541986	-2.857225	4.273631e-03
Litter_depth	-0.19177034	0.056332980	-3.404229	6.635122e-04
Litter_pH	0.69437685	0.259459418	2.676245	7.445230e-03
Mineral_layer_pH20cm_av	-0.64494905	0.195241824	-3.303334	9.554236e-04
K_20cm_av	0.00416044	0.002154184	1.931329	5.344233e-02
C_20cm_av	-0.53766614	0.121198970	-4.436227	9.154931e-06
CN_20cm_av	0.03755102	0.022468110	1.671303	9.466183e-02

B)

	Estimate	Std. Error	t value	Pr(> t)
(Intercept)	0.6369864452	0.5034195521	1.2653192	0.27444465
Elevation	-0.0034998817	0.0013112423	-2.6691342	0.05585631
Stand age	-0.0019840626	0.0022033808	-0.9004628	0.41878665
Field_layer_cover	-0.0006165430	0.0007288586	-0.8459022	0.44524254
litter_depth	-0.0253176398	0.0161531452	-1.5673505	0.19210168
humus_depth	-0.0199376021	0.0117148596	-1.7019070	0.16398807
Mineral_layer_pH20cm_av	-0.1585106997	0.0739029590	-2.1448492	0.09854967
K_20cm_av	0.0014897517	0.0006809124	2.1878757	0.09391455
TotalN_20cm_av	3.3697621372	0.9485901214	3.5523901	0.02374758
Ca_20cm_av	0.0003320045	0.0000825057	4.0240187	0.01581168
C_20cm_av	-0.1919235678	0.0659177140	-2.9115629	0.04360989
CN_20cm_av	0.0600090720	0.0169983735	3.5302832	0.02422438

Table S5. A) Results from the automatic selection of variables in the reduced model to explain variability in pine ectomycorrhizal communities across the 16 stands in this study; B) Relative importance of latitude, litter pH and total soil nitrogen on the pine communities as revealed from the *Adonis* function.

A) Results ordistep:

	Df	Variance	F	Pr(>F)	
latitude	1	0.045706	11.4781	0.001	***
litter pH	1	0.031203	7.8359	0.001	***
TotalN 20cm_av	1	0.009786	2.4574	0.053	.
Residual	12	0.047784			

B) Results Adonis:

	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	
latitude	1	1.9475	1.94755	12.1399	0.39246	0.001	***
litter pH	1	0.8522	0.85217	5.3120	0.17172	0.001	***
TotalN 20cm_av	1	0.2376	0.23765	1.4813	0.04789	0.201	
Residuals	12	1.9251	0.16043		0.38793		
Total	15	4.9625			1.00000		

Significant codes: .0.1 > P > 0.05; *0.05 > P > 0.01; **0.01 > P > 0.001; ***0.001 > P.

Table S6. Single or two species combination selected as indicators of stand type and forest.

1) MIXED FOREST

	A	B	stat	p.value
Tylospora sp2+Thelephoraceae sp1	0.9344	0.6250	0.764	0.025 *

2) NEW FORESTS & THETFORD

NEW FOREST #sps. 28

	A	B	stat	p.value
Elaphomyces granulatus	1.0000	1.0000	1.000	0.001 ***
Russula caerulea	1.0000	1.0000	1.000	0.001 ***
Elaphomyces granulatus+Russula caerulea	1.0000	1.0000	1.000	0.001 ***
Elaphomyces granulatus+Cenococcum geophilum	1.0000	0.8750	0.935	0.003 **
Russula caerulea+Cenococcum geophilum	1.0000	0.8750	0.935	0.003 **
Craterellus tubaeformis	1.0000	0.7500	0.866	0.008 **
Russula ochroleuca+Elaphomyces granulatus	1.0000	0.7500	0.866	0.006 **
Russula ochroleuca+Russula caerulea	1.0000	0.7500	0.866	0.006 **
Elaphomyces granulatus+Lactarius hepaticus	1.0000	0.7500	0.866	0.008 **
Elaphomyces granulatus+Craterellus tubaeformis	1.0000	0.7500	0.866	0.008 **
Elaphomyces granulatus+Tylospora sp1	1.0000	0.7500	0.866	0.011 *
Russula caerulea+Lactarius hepaticus	1.0000	0.7500	0.866	0.008 **
Russula caerulea+Craterellus tubaeformis	1.0000	0.7500	0.866	0.008 **
Russula caerulea+Tylospora sp1	1.0000	0.7500	0.866	0.011 *
Craterellus tubaeformis+Cenococcum geophilum	1.0000	0.7500	0.866	0.008 **
Cenococcum geophilum	0.8071	0.8750	0.840	0.019 *
Cenococcum geophilum+Tylospora sp1	0.8828	0.7500	0.814	0.028 *
Clavulina sp1	1.0000	0.6250	0.791	0.040 *
Russula amethystina	1.0000	0.6250	0.791	0.034 *
Elaphomyces granulatus+Amanita rubescens	1.0000	0.6250	0.791	0.023 *
Elaphomyces granulatus+Clavulina sp1	1.0000	0.6250	0.791	0.040 *
Elaphomyces granulatus+Russula amethystina	1.0000	0.6250	0.791	0.034 *
Russula caerulea+Amanita rubescens	1.0000	0.6250	0.791	0.023 *
Russula caerulea+Clavulina sp1	1.0000	0.6250	0.791	0.040 *
Russula caerulea+Russula amethystina	1.0000	0.6250	0.791	0.034 *
Lactarius hepaticus+Craterellus tubaeformis	1.0000	0.6250	0.791	0.024 *
Craterellus tubaeformis+Tylospora sp1	1.0000	0.6250	0.791	0.035 *
Lactarius hepaticus+Cenococcum geophilum	0.7923	0.7500	0.771	0.036 *

THETFORD FOREST #sps. 9

	A	B	stat	p.value
Amphinema sp1	1.0000	0.7500	0.866	0.011 *
Russula sp2	1.0000	0.7500	0.866	0.007 **
Russula ochroleuca+Russula sp2	1.0000	0.7500	0.866	0.007 **
Thelephoraceae sp1	0.8588	0.7500	0.803	0.030 *
Russula ochroleuca+Amphinema sp1	1.0000	0.6250	0.791	0.018 *
Lactarius hepaticus+Russula sp2	1.0000	0.6250	0.791	0.023 *
Tylospora sp2+Amphinema sp1	1.0000	0.6250	0.791	0.027 *
Amphinema sp1+Russula sp2	1.0000	0.6250	0.791	0.018 *
Tylospora sp2+Thelephoraceae sp1	0.9143	0.6250	0.756	0.046 *

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1				

3) FOREST : STAND TYPE

NEW FOREST : PURE STANDS #sps. 7

	A	B	stat	p.value
Russula ochroleuca+Clavulina sp1	1.0000	0.7500	0.866	0.032 *
Russula caerulea	0.6913	1.0000	0.831	0.009 **
Elaphomyces granulatus+Russula caerulea	0.6363	1.0000	0.798	0.022 *

Russula ochroleuca+Elaphomyces granulatus	0.6251	1.0000	0.791	0.021	*
Russula ochroleuca+Russula caerulea	0.6251	1.0000	0.791	0.021	*
Elaphomyces granulatus+Lactarius hepaticus	0.7326	0.7500	0.741	0.049	*
Russula caerulea+Lactarius hepaticus	0.7326	0.7500	0.741	0.049	*

NEW FOREST : MIXED STANDS #sps. 8

	A	B	stat	p.value	
Lactarius hepaticus+Russula densifolia	1.0000	0.7500	0.866	0.028	*
Craterellus tubaeformis+Russula amethystina	1.0000	0.7500	0.866	0.028	*
Craterellus tubaeformis+Russula densifolia	1.0000	0.7500	0.866	0.028	*
Cenococcum geophilum+Russula densifolia	1.0000	0.7500	0.866	0.028	*
Elaphomyces granulatus+Craterellus tubaeformis	0.6727	1.0000	0.820	0.014	*
Craterellus tubaeformis+Cenococcum geophilum	0.6146	1.0000	0.784	0.021	*
Craterellus tubaeformis	0.5863	1.0000	0.766	0.043	*
Elaphomyces granulatus	0.5560	1.0000	0.746	0.031	*

THETFORD FOREST : PURE STANDS #sps. 7

	A	B	stat	p.value	
Russula ochroleuca+Tricholoma terreum	1.0000	0.7500	0.866	0.034	*
Lactarius hepaticus+Tricholoma terreum	1.0000	0.7500	0.866	0.034	*
Tricholoma terreum+Russula sp2	1.0000	0.7500	0.866	0.034	*
Lactarius hepaticus	0.6556	1.0000	0.810	0.033	*
Lactarius hepaticus+Russula sp2	0.6470	1.0000	0.804	0.010	**
Russula sp2	0.5616	1.0000	0.749	0.021	*
Russula ochroleuca+Russula sp2	0.5616	1.0000	0.749	0.021	*

NEW FOREST : MIXED STANDS #sps. 6

	A	B	stat	p.value	
Tylospora sp2+Thelephoraceae sp1	0.8487	1.0000	0.921	0.004	**
Russula sp1+Thelephoraceae sp1	1.0000	0.7500	0.866	0.034	*
Thelephoraceae sp1	0.7185	1.0000	0.848	0.004	**
Russula sp1	0.9278	0.7500	0.834	0.034	*
Amphinema sp1+Thelephoraceae sp1	0.9195	0.7500	0.830	0.025	*
Russula recondita+Amphinema sp1	0.8722	0.7500	0.809	0.049	*

Significant codes: '0.1 > P > 0.05; *0.05 > P > 0.01; **0.01 > P > 0.001; ***0.001 > P.